Novel pH-Sensitive Polymer-Grafted Magnetic Microparticles for *In Vivo* Sampling of the Murine Small Intestine Microbiome

Elizabeth Ziegler[1], Farnoushsadat Rezaei[2], Bret Ulery[2], Aaron Ericsson[1], Craig Franklin[1], & James Amos-Landgraf[1]

[1]Department of Pathobiology, College of Veterinary Medicine and Mutant Mouse Resource and Research Center,
[2]College of Engineering, University of Missouri, Columbia, MO

Questions on this poster can be directed to lizziegler711@gmail.com

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Background Information

- The microbiome varies between the upper gastrointestinal tract (GIT) and the lower GIT
- Our current methods of measuring the intestinal microbiome rely on fecal testing and thus heavily reflect the lower GIT
- Research has shown that the number of bacteria in the colon is higher than the number of bacteria in the small intestine
- The pH varies along the different segments of the GIT (see Table 1)

Table 1: The pH values of various segments of the mammal GIT

Section of Gastrointestinal Tract	pH
Stomach	1.5-3.5
Duodenum	5.5-6.0
Jejunum	6.0-7.4
Ileum	7.4

Objective and Significance

- The objective of this study is to use a novel, proprietary magnetic polymer-coated bead to capture bacteria in the small intestine in a live mouse model
- The significance of this study is to discover the role of the small intestinal microbiome in various disease states, including dysbiosis, intestinal diseases, and even epigenetic factors

Methodology

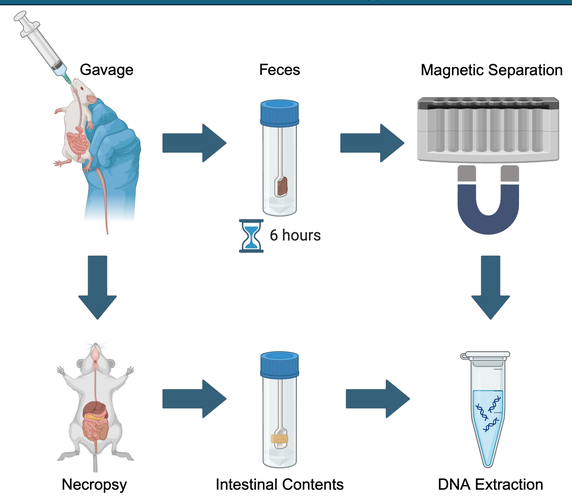

Figure 1: Schematic of methods that demonstrates mice given magnetic polymer-coated beads via oral gavage (n=4). Fecal samples were collected before, at, and after the 6 hour mark post gavage and were magnetically separated. The mice underwent necropsy and intestinal contents were collected from the esophagus, stomach, small intestine, cecum, and colon. DNA was extracted from all the samples and submitted for sequencing. Schematic designed with BioRender.

Histidine Polymer Beads

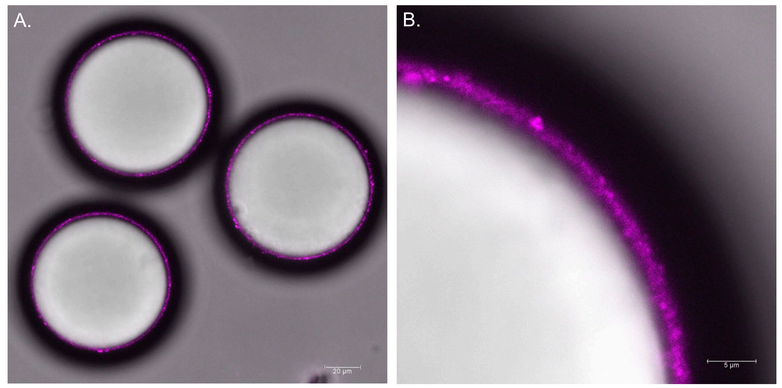

Figure 2: Images of the magnetic polymer-coated beads showing captured bacteria. WGA was used to stain bacteria (magenta). (**A**) At 100X with a 20 um scale bar (**B**) Zoomed in using confocal microscopy with a 5 um scale bar

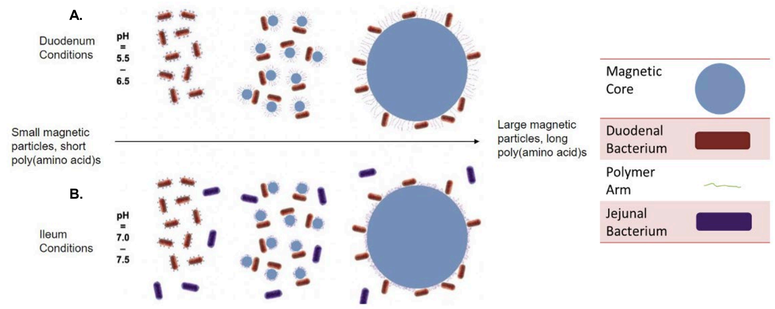

Figure 3: Depiction of the magnetic polymer-coated beads capturing bacteria. The histidine polymer arms are attached to a magnetic bead central core in order to be isolated using a magnet. A small magnetic bead is depicted in the middle segment of the image and a large magnetic bead is depicted in the right side of the image. (**A**) In the acidic conditions of the duodenum (pH = 5.5-6.5), the histidine polymer arms are in the reduced conformation and contain two positively charged nitrogen groups. In this state, the polymer arms will allow bacteria in the duodenum to attach to them. (**B**) In the neutral conditions of the Ileum (pH = 7.0-7.5), the histidine polymer arms are in the oxidized conformation and contain two neutrally charged nitrogen groups. In this state, the polymer arms hold onto the bacteria that were attached to them from the duodenum, while excluding bacteria that is native to the jejunum and ileum.

Acknowledgements

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Results

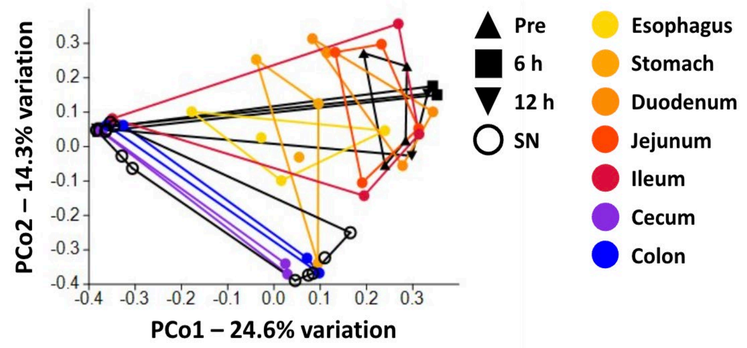

Figure 4: Principal coordinate analysis (PCoA) plot showing Jaccard dissimilarities between capture beads isolated from feces prior to 6 hour (Pre), and at 6 and 12 hours post-administration, the supernatant (SN) from which the beads were separated, and samples collected from different regions of the gastrointestinal tract post-mortem.

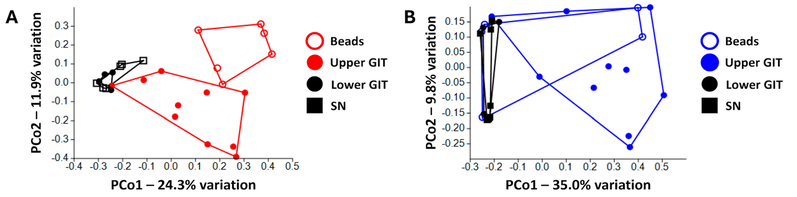

Figure 5: Principal coordinate analysis (PCoA) plot showing Jaccard dissimilarities between capture beads isolated from GM^{low} (A) or GM^{high} (B) feces at 5.5 to 6.5 hours post-administration, the supernatant (SN) from which the beads were separated, and samples collected from upper and lower gastrointestinal tract (GIT) postmortem.

Conclusions

- The pH-sensitive polymer grafted particles are readily passed through the GIT
- These beads can be magnetically isolated from feces
- The magnetic polymer-coated beads are capturing bacteria from the small intestine, while excluding bacteria from the large intestine
- Beads isolated from both GM^{low} and GM^{high} mice show binding of small intestinal bacteria

Future Goals

- Measure the transit time of the magnetic polymer-coated beads through the mouse GIT
- Create an experiment testing the accuracy of the conformation change of the polymer arms at the targeted pH levels
- Analyze the inoculated magnetic polymer-coated beads for any level of bacterial contamination